Apostle MiniMax™ High Efficiency cfDNA Isolation Kit Manual (Standard Edition), 1 mL x 10 Preps

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Product description

The Apostle MiniMax™ High Efficiency cfDNA Isolation Kit is designed for isolation of DNA from cell free plasma, serum, urine samples. The kit uses proprietary Apostle MiniMax™ technology, offers highly efficient, reproducible recovery of high-quality cfDNA with high yield. The isolated DNA samples is suitable for a broad range of subsequent applications, including sequencing, PCR, etc.

Kit capacity

The kit is capable of cfDNA isolation for 1 mL x 10 samples.

Kit contents and storage condition

Contents	Amount	Storage
Magnetic Nanoparticles	0.165 mL	Room temperature, in the dark
Proteinase K	1.0 mL	
Sample Lysis Buffer	1.1 mL	
cfDNA Lysis/Binding Solution	13.75 mL	
cfDNA Wash Solution	22 mL	
cfDNA 2nd Wash Solution	5.5 mL	
cfDNA Elution Solution	1.5 mL	

Note: Magnetic nanoparticle solution should be brown solution. Vortex magnetic nanoparticle solution to fully resuspend the nanoparticles before use.

Sample Lysis Buffer, cfDNA Lysis/Binding Solution, and cfDNA Wash Solution should be clear solution. If precipitate is observed in any of these reagents, warm the solution to 37 °C until the precipitate dissolves.

Read SDS before use. DO NOT ADD acids or bleach to any liquid wastes containing this product. Use ethanol if necessary.

Required materials not supplied

Adjustable micropipettes (1 mL, 200 uL, 20 uL) and tips
Magnets (specifically designed for 15 mL and 2 mL tubes)
Table top centrifuge
Non-stick, low-binding, DNase/RNase-free tubes (1.5 mL and 15 mL)
Vortex/Shaker
Ethanol (200 Proof)
Heater (for Sample Lysis Buffer)

Procedure for manual isolation of cfDNA

A. Sample lysis

1. Add components to a 15 mL tube **in the order** indicated below, based on volume of sample.

Reagents	Plasma/serum volume		
	1 mL	2 mL	5 mL
Proteinase K	40 uL	80 uL	200 uL
Plasma/serum	1 mL	2 mL	5 mL
Sample Lysis Buffer	100 uL	200 uL	500 uL

Caution: avoid mixing proteinase K with Sample Lysis Buffer before Plasma/serum.

2. Mix the solution well by vortexing briefly and incubate the mixture at 60 °C for 20 minutes.

3. At the end of the incubation, cool the tubes containing the plasma/serum to room temperature.

B. Bind cfDNA to magnetic nanoparticles

4. Prepare the binding/nanoparticle solution according to the table below, and mix well (**Note**: equilibrate the Apostle MiniMax™ Magnetic Nanoparticles vial (**Green Cap**) to room temperature and then vortex to fully resuspend the nanoparticles before use):

Reagents	Plasma/serum volume		
	1 mL	2 mL	5 mL
cfDNA Lysis/Binding Solution	1.25 mL	2.5 mL	6.25 mL
Magnetic Nanoparticles	15 uL	30 uL	75 uL

5. Add the prepared binding/nanoparticle solution to the plasma/serum sample, thoroughly mix by vortexing briefly, or invert the tube 10 times (**Note**: avoid excessive vortexing, which generate excessive bubbles).

6. Shake at moderate-high speed for 10 minutes to bind the cfDNA to the nanoparticles.

7. Place the tube on magnet for 5 min, or until the solution clears and the beads are pelleted against the magnet.

8. Carefully remove the supernatant (e.g. using pipette to remove supernatant, or discard the supernatant with the existence of the magnet to attract nanoparticles).

C. Wash with Apostle MiniMax™ cfDNA Wash Solution

9. Remove the tube (referred to as lysis/binding tube below) from the magnet, add 1 mL of Apostle MiniMax™ cfDNA Wash Solution, vortex to resuspend the nanoparticles.

10. Transfer the magnetic nanoparticle suspension to a new non-stick 1.5 mL microcentrifuge tube, and save the lysis/binding tube.

11. Place the 1.5 mL tube on magnet to pellet the nanoparticles for 1 min.

12. Use the supernatant in the 1.5 mL tube to rinse the saved lysis/binding tube, and transfer any residual nanoparticles to the 1.5mL tube, discard the lysis/binding tube.

13. Place the 1.5 mL tube on magnet for 2 min, or until the solution clears and the nanoparticles are pelleted against the magnets.

14. Remove the supernatant carefully using pipette.

15. Remove the 1.5 mL tube from the magnet, add 1 mL of Apostle MiniMax™ cfDNA Wash Solution, then vortex for 30 seconds.

16. Centrifuge the 1.5 mL tube using tabletop centrifuge briefly to bring solution to the bottom, place the 1.5 mL tube on magnet for 2 min, or until the solution clears and the nanoparticles are pelleted against the magnets.

17. Remove the supernatant carefully using pipette.

D. Wash with Apostle MiniMax™ cfDNA 2nd Wash Solution

18. Pre-dilute Apostle MiniMax™ cfDNA 2nd Wash Solution 1:4 in Ethanol before use, to a final composition of 20% Apostle MiniMax™ cfDNA 2nd Wash Solution and 80% Ethanol.

    The amount of final secondary wash buffer required is 2mL per sample.

19. Remove the 1.5 mL tube from the magnet, add 1 mL of the prepared secondary wash buffer (20% Apostle MiniMax™ cfDNA 2nd Wash Solution, with 80% Ethanol), then vortex for 30 seconds.

20. Centrifuge the 1.5 mL tube using tabletop centrifuge briefly to bring solution to the bottom, place the 1.5 mL tube on magnet for 2 min, or until the solution clears and the nanoparticles are pelleted against the magnets.

21. Remove the supernatant carefully using pipette.

22. Repeat step 19-21 for a second wash.

23. Remove the 1.5 mL tube from the magnet, centrifuge the 1.5 mL tube using tabletop centrifuge briefly to bring all liquid to the bottom, place the 1.5 mL tube on magnet, until the solution clears and the nanoparticles are pelleted against the magnets.

24. Remove any liquid left in the bottom of 1.5 mL tube.

25. Keep the 1.5 mL tube on the magnet, air dry the nanoparticles for 3 minutes. (When environment humidity is high, time can be longer to minimize the residual amount of ethanol, which will affect elution efficiency.)

E. Elute cfDNA from magnetic nanoparticles

26. Remove the 1.5 mL tube from the magnet, add Apostle MiniMax™ cfDNA Elution Solution (**Blue Cap**) to the 1.5 mL tube according to the following table, based on initial sample volume.

Plasma/serum volume	1 mL	2 mL	5 mL
Suggested cfDNA Elution Solution Volume	20 uL	40 uL	100 uL

27. Vortex the 1.5 mL tube to resuspend the magnetic nanoparticles in the solution, then vortex for another 5 minutes to elute the cfDNA from the nanoparticle.

28. Centrifuge the 1.5 mL tube using tabletop centrifuge briefly to bring solution to the bottom, place the 1.5 mL tube on a magnet, until the solution clears and the nanoparticles are pelleted against the magnets.

29. Collect the supernatant that contains cfDNA in a nonstick, DNase and RNase free microcentrifuge tube.

30. Store the cfDNA sample at 4 °C for short term storage, and -20 °C for long term storage.

31. If isolated cfDNA sample characterization and quantification is needed, it is recommended to use Bioanalyzer 2100 + High Sensitivity DNA Analysis Kit, due to its low detection limit (5 pg/uL).